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(54) Substrate for array printing

(57) A substrate for use in supporting high density biological or chemical arrays that is made from borosilicate or boroaluminosilicate glass. It has been demonstrated that a functionalized coating used to immobilize oligonucleotides for example, retains its functionality when exposed to environmental stresses when it is applied to a slide composed of a glass material having a low sodium oxide content.

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Description

Field Of Invention

[0001] The invention relates to high density biological and chemical arrays and specifically to an improved substrate material onto which arrays are deposited.

Background of Invention

[0002] Oligonucleotide hybridization is widely used to determine the presence in a nucleic acid of a sequence that is complimentary to the oligonucleotide probe. In many cases, this provides a simple, fast, and inexpensive alternative to conventional sequencing methods. Hybridization does not require nucleic acid cloning and purification, carrying out base-specific reactions, or tedious electrophoretic separations. Hybridization of oligonucleotide probes has been successfully used for various purposes, such as analysis of genetic polymorphisms, diagnosis of genetic diseases, cancer diagnostics, detection of viral and microbial pathogens, screening of clones, genome mapping and ordering of fragment libraries.

[0003] An oligonucleotide array is comprised of a number of individual oligonucleotide species tethered to the surface of a solid support in a regular pattern, each one in a different area, so that the location of each oligonucleotide is known. An array can contain a chosen collection of oligonucleotides, e.g., probes specific for all known clinically important pathogens or specifics for all known sequence markers of genetic diseases. Such an array can satisfy the needs of a diagnostic laboratory. Alternatively, an array can contain all possible oligonucleotides of a given length *n*. Hybridization of a nucleic acid with such a comprehensive array results in a list of all its constituent *n*-mers, which can be used for unambiguous gene identification (e.g., in forensic studies), for determination of unknown gene variants and mutations (including the sequencing of related genomes once the sequence of one of them is known), for overlapping clones, and for checking sequences determined by conventional methods. Finally, surveying the *n*-mers by hybridization to a comprehensive array can provide sufficient information to determine the sequence of a totally unknown nucleic acid.

[0004] Oligonucleotide arrays can be prepared by synthesizing all the oligonucleotides, in parallel, directly on the support, employing the methods of solid-phase chemical synthesis in combination with site-directing masks as described in US Pat. 5,510,270. Using an efficient photolithographic technique, miniature arrays containing as many as 10^5 individual oligonucleotides per cm^2 of area have been demonstrated.

[0005] Another technique for creating oligonucleotide arrays involves precise drop deposition using a piezoelectric pump as described in US Pat. 5,474,796. The piezoelectric pump delivers minute volumes of liquid to a substrate surface. The pump design is very similar to the pumps used in ink jet printing. This picopump is capable of delivering 50 micron and 65 picoliter droplets at up to 3000 Hz and can accurately hit a 250 micron target. When energized, a microdroplet is ejected from the pump and deposited on the array plate at a functionalized binding site.

[0006] Further approaches to forming an array involve repeatedly contacting a substrate surface with typographic pins holding droplets and using ink jet printing mechanisms to lay down an array matrix.

[0007] In choosing a substrate for use as a support for the attachment of oligonucleotides, several characteristics must be considered. First, the surface must be compatible with the method of detection of hybridization. Spectroscopic, chemiluminescent and fluorescent detection techniques are the detection techniques of choice for DNA research involving high density arrays. In order to use these techniques, it is desirable that the substrate be optically transparent. A second important characteristic is that the linkage of the penultimate oligonucleotide to the surface have high chemical stability, at least equal to that of the polyphosphate backbone in DNA.

[0008] The substrates that support the arrays are conventionally 1 by 3 inch slides made from soda lime glass and coated with a polar silane which contains for example an amino group suitable for anchoring solid phase oligonucleotide synthesis, and specifically for cross linking the DNA molecules. Patterned derivitization may be achieved on this surface by photoresist or masking techniques. In this way, patterned wetting sites can achieved on an otherwise nonwetting surface as well as patterned functionalized sites on an otherwise nonfunctionalized surface.

[0009] One problem with the conventional use of soda lime glass as a substrate for the support of high density arrays is the presence of particulate contamination that is common in the production of such low grade glass. Particulate contamination is of special concern while dealing with samples on such small scale as 10,000 target sites per slide. Further, the sodium contained in soda lime glass can be easily mobilized to exit the glass. Hazing is a result which negatively affects the transparency of the glass and consequently disturbs the detection techniques previously mentioned. Finally, it is difficult to obtain a uniform functionalized coating, such as an amino functional silane coating, on the surface of the slides now in conventional use. Without a uniform coating, oligonucleotide attachment is uneven, leading to varied and unreliable detection results.

Summary of Invention

[0010] An improved substrate for use in the printing or the synthesis of biological and chemical arrays is disclosed. The substrate is a substantially flat support made from a borosilicate or boroaluminosilicate glass.

Detailed Description of the Invention

[0011] The functionalized coating of the surface of glass substrates with amino functionalized amines, for example, is the backbone of high density array manufacture. A substantially even coating of the functionalized coating, as discussed above, is required. It has been discovered that using a known glass that can be manufactured by known methods to obtain a specific smoothness has important uses as a biological substrate.

[0012] The substrate that is the subject of the present invention, which preferably takes the form of a 1 inch x 3 inch slide, is made from a borosilicate or boroaluminosilicate glass, and more preferably from Conning Incorporated 1737 LCD glass, consisting essentially, expressed in terms of mole percent, of

SiO_2	67.6	BaO	4.31
Al_2O_3	11.4	MgO	1.31
B_2O_3	8.53	SrO	1.29
CaO	5.2	As_2O_3	0.39

[0013] The slides may be cut from a sheet of glass that has preferably been formed by the fusion draw process disclosed in United States Patents 3,338,696 and 3,682,609, incorporated herein by reference. This process provides for the manufacture of high liquidus viscosity glasses, such as borosilicate or boroaluminosilicate glasses, in sheets having extreme smoothness. Although the borosilicate or boroaluminosilicate sheets may be manufactured by other methods and subsequently polished, using the fusion draw process is preferred as polishing steps in manufacture lead to the potential for particulate contamination on the substrate surface. In another preferred embodiment, the slide is made from Corning Incorporated 7059 LCD glass. At any rate, the preferred glass composition of the substrate slide will have a weight percent of less than 15% sodium oxide, or any other alkali metal oxide. Several suitable glass compositions are listed in commonly assigned United States Patent 5,374,595.

[0014] It is further preferred that the slide have a uniform surface smoothness such that the average roughness (Ra) of the top surface, as taken on a 20 micron by 20 micron scan employing an atomic force microscope (AFM), is less than 10 nanometers, and preferably less than 10 angstroms. The top surface is the portion of the slide in which the binding entity array is preferably synthesized, deposited, or otherwise attached. Even more preferably, the average roughness is less than 5 angstroms. The fusion draw flat glass forming method as disclosed in U.S. Pat. 3,338,696 and 3,682,609 when used to produce LCD 1737 glass, for example, provides a surface having the preferred average roughness of less than 5 angstroms. Alternatively, the preferred average roughness may be achieved by polishing. The smoothness of the surface helps enable the application of a uniform surface coating.

[0015] The coating that is preferably-applied to the borosilicate or aluminosilicate substrate for use in oligonucleotide immobilization is a polar silane which contains for example an amino group suitable for anchoring solid phase oligonucleotide synthesis, and specifically for cross linking the DNA molecules. Alternatively, the polar silane may contain a hydroxyl after hydrolysis (before hydrolysis, this group is preferably an alkoxy group). Suitable coatings include functionalized alkoxysilane or chlorosilane whereby the silane has between 1 and 3 alkoxy or chlorine groups. Further, the top surface may have patterned derivitization through the use of photoresist or masking techniques, for example.

[0016] Use of the borosilicate or boroaluminosilicate substrate is not limited to use with amine functionalized coatings for oligonucleotide array support. The substrate may be used as a solid support for any of a variety of binding entities which include any biological or synthetic molecule having a specific affinity for another molecule, through covalent bonding or non-covalent bonding. Preferably, a specific binding entity contains (either by nature or by modification) a functional chemical group (primary amine, sulfhydryl, aldehyde, carboxylic, acrylic, etc.), a common sequence (nucleic acids), an epitope (antibodies), a hapten, or a ligand, that allows it to covalently react or non-covalently bond to a common function group on the surface of a substrate. Specific binding entities include, but are not limited to: deoxyribonucleic acids (DNA), ribonucleic acids (RNA), synthetic oligonucleotides, antibodies, proteins, peptides, lectins, modified polysaccarides, synthetic composite macromolecules, functionalized nanostructures, synthetic polymers, modified/blocked nucleotides/nucleosides, modified/blocked amino acids, fluorophores, chromophores, ligands, chelates and haptens.

Table 2 (continued)

Compound	Mole Percent (%)
TiO_2	0.006
Fe_2O_3	0.07
As_2O_3	0.015

Table 3

Compound	Mole Percent (%)
SiO_2	65
Na_2O	6.4
K_2O	6.6
Al_2O_3	4.1
TiO_2	4.2
B_2O_3	8.1
ZnO	5.6
Sb_2O_3	0.2

[0024] Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

Claims

1. A substrate for use in supporting high density biological or chemical arrays comprising a substantially flat 1" by 3" slide comprised of a borosilicate or a boroaluminosilicate glass having a sodium oxide content of less than 12 mole percent.

2. The substrate of claim 1 further having a top surface having an average roughness of less than 10 nanometers.

3. A method of using a substrate for high density biological or chemical arrays comprising the steps of:

   a) providing a substantially flat slide comprised of a glass material having a sodium oxide content of less than 12 mole percent, and
   b) attaching a binding entity array to said slide.

4. The method of claim 12 further comprising the step of applying a coating of a functionalized polar silane to at least a portion of a surface of said material prior to said attaching step.

5. The method of claim 12 wherein said slide has a top surface having an average roughness of less than 10 nanometers.

6. The method of claim 13 wherein said polar silane contains an amine group.

7. The method of claim 13 wherein said polar silane is aminopropyl triethoxysilane.

8. The method of claim 12 wherein said glass material is a boroaluminosilicate glass.

9. The method of claim 12 wherein said glass material is a borosilicate glass.

Example

[0017] A comparison study was performed to determine the durability of an identical coating applied 1"x 3" slides made of three different glasses: soda-lime glass, borosilicate glass, and boroaluminosilicate glass. A coating of gamma-aminopropyl triethoxysilane was applied to each of the slides to be tested. The slides were then immersed in boiling water for a period of time ranging from 0.5 to 5 hours. When the aminated coating is retained on the surface of the slide after exposure to certain environmental stresses (in this instance, boiling water), the functionality of the surface is said to have retained its functionality and the result of the test for durability is positive.

[0018] The durability of the aminated coating was measured by using a staining test based on a Au/Ag growth process. This process reveals the presence of amine functions on the substrate surface. When Au/Ag growth occurs, the test is positive for the presence of amine functions. A positive test is indicated by visual observation of a dense and uniform metal gray coating. A substrate free of amine functionality does not stain and remains clear.

[0019] The staining process test was conducted as follows: The slides were dipped in AURODYE FORTE RPN 490 (Amersham Life Science, Amersham International) for 1 hour. The slides were then rinsed with pure water. The slides were then dried with N_2. The slides were next dipped in INTENSE BL SILVER ENHANCEMENT SOLUTION RPN 492 (Amersham Life Science Amersham International) for 5 minutes. The slides were then again rinsed with pure water and dried with N_2. The presence or absence of a metal gray coating was determined by visual observation.

[0020] As mentioned, substrates made of three different materials, namely soda lime glass, borosilicate glass, and boroaluminosilicate glass, were tested. Table 1 shows the length of exposure in boiling water required for the gamma-aminopropyl triethoxysilane coated slide to lose its amine functionality (time required for the staining test to read negative).

Table 1

Glass Substrate	Coating Durability (in hours)
Soda-Lime Glass	0.5
Borosilicate Glass	2.0
Boroaluminosilicate Glass (1737 LCD Glass)	4.0

[0021] The results shown in Table 1 demonstrate that the durability of the gamma-aminopropyl triethoxysilane coating on borosilicate or boroaluminosilicate glasses is far superior to that of the same coating on soda-lime glass.

[0022] Although not intending to be bound by the explanation, it is thought that the lower or nonexistent levels of sodium oxide in the samples of borosilicate and boroaluminosilicate glass provide the advantageous durability characteristics shown in the testing. Preferably, the glass material as used for the high density assay substrate has a sodium oxide content ofless than 12 mole percent, and even more preferably less than 8 mole percent, and more preferably still, no sodium oxide content at all. For this reason it may be contemplated, as an alternative, to use any glass that has this requisite sodium oxide content including aluminosilicate glass, for example.

[0023] The composition of soda-lime glass is given as an example in Table 2. The composition of borosilicate glass, as used in this example, is given in Table 3. The composition of 1737 LCD glass, the boroaluminosilicate glass used in this example, is given above.

Table 2

Compound	Mole Percent (%)
SiO_2	71.5
Na_2O	13.3
K_2O	0.3
CaO	8
MgO	4.1
Al_2O_3	1.5
SO_3	0.37

10. A substrate for use in supporting high density biological and chemical arrays comprising a glass material having a sodium oxide content of less than 12 mole percent and having a top surface having an average roughness of less than 10 nanometers.

11. A substrate for use in supporting high density biological and chemical arrays comprising:

a glass material having a sodium oxide content less of than 12 mole percent and having a top surface having an average roughness of less than 10 nanometers
a functionalized coating of a polar silane covering at least a portion of a surface of said material; and
said substrate characterized in that the functionality of said coating is retained after immersion in boiling water for an amount of time exceeding 1 hour.

12. The substrate of claim 20 wherein said material has an average roughness of less than 5 angstroms.

13. The substrate of claim 20 wherein said sodium oxide content is less than 8 mole percent.

14. The substrate of claim 20 wherein said polar silane contains an amine group.

15. The substrate of claim 20 wherein said polar silane contains at least one hydroxyl group.

16. The substrate of claim 20 wherein said polar silane contains at least one alkoxy group.

17. The substrate of claim 20 wherein said polar silane contains at least one chlorine group.

18. The substrate of claim 20 wherein said polar silane is aminopropyl triethoxysilane.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 98 40 0242

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
D,X	US 5 474 796 A (T.M. BRENNAN) 12 December 1995 * claims *	1,3-9	B01J19/00 C03C3/089 C03C17/30
X	EP 0 035 719 A (UNIVERSITY PATENTS) 16 September 1981 * page 4, line 7 - line 16 * * page 20, line 28 - page 21, line 7 *	1,3-9	
X	WO 93 09668 A (AFFYMAX TECHNOLOGY NV) 27 May 1993 * page 20, line 16 - line 25 * * page 31, line 43 - page 32, line 43 *	1,3-9	
D,A	US 5 374 595 A (W.H. DUMBAUGH ET AL.) 20 December 1994 * abstract *	1-18	
A	CHEMICAL ABSTRACTS, vol. 111, no. 20, 13 November 1989 Columbus, Ohio, US; abstract no. 177392, page 201; XP002070218 * abstract * & CS 257 226 A (M. CAPKA) 15 February 1989	1-18	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)
B01J
C03C

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	2 July 1998	Reedijk, A

CATEGORY OF CITED DOCUMENTS

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